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Presumptive Tests

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The necessary characteristics of presumptive tests are that they require no more than weeks or months to perform; the results from such tests be highly reproducible; the significance of test results be validated by empirical correlation; they be capable of being coupled to appropriate metabolic activation systems; they circumvent the requirement that the intact animal (or a similarly limited condition) serve as the statistical unit of the experiment. Currently, most but not all presumptive tests utilize individual cells, either microbial or mammalian cells in tissue culture, which are grown, treated, and analyzed *in vitro*.

There are three major categories of presumptive tests which have been considered useful as predictors of carcinogenesis or mutagenesis. The first category includes those tests which assess the potential of a substance and/or its metabolite to damage and/or interact with DNA. The rationale for tests of this type is that DNA is known to be the principal target molecule for mutagenesis and that a common feature of carcinogens is their ability to form electrophiles and to react with molecules such as DNA to form new covalent bonds. Among the tests which measure either directly or indirectly damage to DNA are: liquid and thin-layer chromatography of the constituent nucleotides of DNA; use of isotopically labeled reactants; repair-replication of DNA.

The next category of presumptive tests are the mutational assay systems in bacteria (such as the Ames test), other microbial organisms and mammalian cells in tissue culture. These tests also reflect whether damage to DNA has occurred by interaction with a chemical or its metabolic product. This entire category can be subdivided into two major classes: those tests responding only to highly specific chemical changes on the DNA (e.g., specific base-substitution); and those which are to varying degrees nonspecific, capable of responding to virtually any type of damage.

The last category of presumptive tests in neoplastic tranformation, which includes among others the following specific systems: BALB/3T3 cells; Fischer rat embryo cells; Fischer rat embryo cells infected with Rauscher leukemia virus; hamster embryo cells (transplacental exposure). These tests measure, in normal cells in tissue culture, cellular transformation to neoplasia. Such cells when transplanted into their appropriate host animals grow and exhibit all of the properties expected of cancer cells.

The utility of presumptive tests is likely to go far beyond their current perceived usefulness as a preliminary screen for further, more in-depth, testing. Presumptive tests will eventually prove useful in providing additional evidence for deciding upon marginal data on the carcinogenicity or mutagenicity of a chemical derived from animal experiments and in studying the interaction between two or more substances. Additionally, they should prove useful as research tools for investigating the molecular mechanism of action of carcinogens; for identifying the molecular targets of carcinogenicity; and in determining what specific events are obligatory to the process of carcinogenesis. As knowledge from the above studies accumulates, it is expected that the presumptive tests will provide useful information on species-to-species extrapolations and, with certain of the tests, dose-response information useful in the extrapolation of data from high dose to low.

While the current status of the above presumptive tests is, in part, subject to opinion, certain facts appear indisputable. Direct chemical methods for detecting damage to DNA are underutilized, but it must be recognized that the applicability of these methods is dependent upon the particular chemical class in question. Methods for assessing DNA repair-replication in mammalian cells appear, at present, to be more useful as a research tool than as a prescreen test. Among the many gene mutational assay systems available, experience is greatest with

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the several tester strains in *Salmonella* developed by Dr. Bruce Ames. In fact, several hundred substances have already been tested in these strains, and an excellent correlation with certain classes of chemical carcinogens has been reported. Of the mammalian tissue culture systems, the L-5178-Y mouse lymphoma cell line is currently the most widely used and appears the easiest to handle with facility.

In general, repair and mutational assay systems are more highly developed, more widely used, and their results easier to reproduce in different laboratories than in the case for those cell culture assays which detect neoplastic transformation. Current efforts in the development of assays for neoplastic transformation are focusing on standardization of methodology, assessment of reproducibility, improving sensitivity, developing endpoints other than morphological transformation, and providing adequate metabolic activation systems.